

#8



SEQUENCE LISTING

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<120> REDUCING IMMUNOGENICITIES OF IMMUNOGLOBULINS BY FRAMEWORK-PATCHING

<130> 655

<140> US 09/892,613

<141> 2001-06-27

<160> 32

<170> PatentIn version 3.1

<210> 1

<211> 369

<212> DNA

<213> Artificial Sequence

<220>

<223> FR-patched heavy chain variable region sequence (Full DNA Sequence) formed by joining the N- and C- terminal (SEQ 3 and 6) halves at the KpeI site.

<220>

<221> V_region

<222> (1)..(369)

<223>

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<400> 1
gaagtgcagc tgctggagtc tgggggaggc ttagtgcagc ctggagggtc cctgaggctc      60
tcctgtgcag cctctggatt ctccctcagc atctatgaca tgtcttgggt tcgccaggca      120
ccgggaaagg ggctggagtg ggtcgcatac attagtagtg gtggtggtac cacctactat      180
ccagacactg tgaagggccg attcaccatc tccagagaca atgccaagaa ctccctgtac      240
ctgcaaatga acagtctgag ggtggaggac acagccttat attactgtgc aagacatagt      300
ggctacggta gtagctacgg ggttttgttt gcttactggg gccaaaggac tctgggtcact      360
gtctcttca

```

<210> 2

<211> 123

<212> PRT

<213> Chimaera sp.

<400> 2

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Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1      5      10
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Ser Ile Tyr
20     25     30
Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35     40     45
Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val
50     55     60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65     70     75     80
Leu Gln Met Asn Ser Leu Arg Val Glu Asp Thr Ala Leu Tyr Tyr Cys
85     90     95

```

Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr
 100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 3
 <211> 111
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> N-template is a synthetic sense-strand oligonucleotide encoding a
 amino acid 14-50 of the VH region (SEQ ID No. 2). The template is
 PCR-amplified by two primers (SEQ ID No. 4 and 5)

<220>
 <221> V_region
 <222> (1)..(111)
 <223>

<400> 3
 cctggagggt ccctgaggct ctctgtgca gcctctggat tctccttcag tatctatgac 60
 atgtcttggg ttgccaggc accgggaaag gggctggagt gggtcgcata c 111

<210> 4
 <211> 57
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am
 ino acid 1-19 of the VH region (SEQ ID No. 2). The 3' end of the
 primer overlaps with the 5' end of the template by 18 nucleotides

<220>
 <221> primer_bind
 <222> (1)..(57)
 <223>

<400> 4
 gaagtgcagc tgctggagtc tgggggaggc ttagtgcagc ctggagggtc cctgagg 57

<210> 5
 <211> 48
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encodi
 ng amino acid 43-59 of the VH region(SEQ ID No. 2). The primer o
 verlaps with the template by 21 nucleotides.

<220>
 <221> primer_bind
 <222> (1)..(48)
 <223>

<400> 5
 gtaggtggta ccaccaccac tactaatgta tgcgaccac tccagccc 48

<210> 6
 <211> 132
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C-terminal is a synthetic sens -strand oligonucleotid encoding a

mino acid 68-111 of the VH region (SEQ ID No 2) The t mplate is P
CR-amplified by two primers (SEQ ID No 7 and 8)

<220>
<221>
<222>
<223>

V_region
(1)..(132)

<400> 6
ttcacatct ccagagacaa tgccaagaac tccctgtacc tgcaaataaa cagtctgagg 60
gtggaggaca cagccttata ttactgtgca agacatagtg gctacggtag tagctacggg 120
gttttggttg ct 132

<210> 7
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 55-74 of the VH region (SEQ ID No 2). The 3' end of the primer overlaps with the 5' end of the template by 21 nucleotides

<220>
<221> primer_bind
<222> (1)..(60)
<223>

<400> 7
ggtaggtacca cctactatcc agacactgtg aagggtccgat tcacatctc cagagacaaat 60

<210> 8
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 105-123 of the VH region (SEQ ID No 2). The primer and the template overlaps by 21 nucleotides.

<220>
<221> primer_bind
<222> (1)..(57)
<223>

<400> 8
tgaagagaca gtgaccagag tcccttggcc ccagtaagca aacaaaaccc cgtagct 57

<210> 9
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> FR-patched light chain variable region sequence formed by joining the N- and C- terminal (SEQ 11 and 14) halves at the KpeI site.

<220>
<221> V_region
<222> (1)..(321)
<223>

<400> 9
gatatccaga tgaccagtc tccatcctcc ctgtctgcct ctgtgggaga cagagtcacc 60
attagttgca gggcaagtca ggacattagc aattatttaa actggtatca gcagaaacca 120

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ggtaaggctc cgaaactcct gatctactac actagtatat tacactcagg agtcccatca      180
aggttcagtg gcagtggggc tggaacagaa ttactctca ccattagctc cctgcagcca      240
gaagattttg ccacttactt ttgccaacag ggtaatacgc ttccgtggac gttcggtgga      300
ggcaccaagg tggaaatcaa a                                              321

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<210> 10
<211> 107
<212> PRT
<213> Chimaera sp.

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```
<400> 10
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```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1      5      10      15

```

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Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
20     25     30

```

```

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35     40     45

```

```

Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50     55     60

```

```

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65     70     75     80

```

```

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
85     90     95

```

```

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100    105

```

```

<210> 11
<211> 108
<212> DNA
<213> Artificial Sequence

```

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<220>
<223> N-template is a synthetic sense-strand oligonucleotide encoding a
      mino acid 11-46 of the VL region (SEQ ID No. 10). The template is
      PCR-amplified by two primers (SEQ ID No. 12 and 13)

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<220>
<221> V_region
<222> (1)..(108)
<223>

```

```

<400> 11
ctgtctgcct ctgtgggaga cagagtcacc attagttgca gggcaagtca ggacattagc      60
aattatttaa actggtatca gcagaaacca ggtaaggctc cgaaactc                  108

```

```

<210> 12
<211> 51
<212> DNA
<213> Artificial Sequence

```

```

<220>
<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am
      ino acid 1-17 of the VH region (SEQ ID No 10). The 3' end of the
      primer overlaps with the 5'end of the template by 21 nucleotides

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```
<220>
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<221> primer_bind
 <222> (1)..(51)
 <223>

<400> 12
 gatatccaga tgaccacgctc tccatcctcc ctgtctgcct ctgtgggaga c 51

<210> 13
 <211> 40
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 40-53. The primer and the template overlaps by 18 nucleotides.

<220>
 <221> primer_bind
 <222> (1)..(40)
 <223>

<400> 13
 atatactagt gtagtagatc aggagtttcg gagccttacc 40

<210> 14
 <211> 120
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C-terminal is a synthetic sense-strand oligonucleotide encoding amino acid 59-98 of the VH region (SEQ ID No 10) The template is P CR-amplified by tow primers (SEQ ID No 15 and 16)

<220>
 <221> V_region
 <222> (1)..(120)
 <223>

<400> 14
 ccatcaaggc tcaagtggcag tgggtctgga acagaattta ctctcacat tagctccctg 60
 cagccagaag attttgccac ttacttttgc caacagggta atacgcttcc gtggacgttc 120

<210> 15
 <211> 49
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 50-65 of the VH region (SEQ ID No. 10). The 3' end of the primer overlaps with the 5' end of the template by 21 nucleotides

<220>
 <221> primer_bind
 <222> (1)..(49)
 <223>

<400> 15
 ctacactagt atattacact caggagtccc atcaagggtc agtggcagt 49

<210> 16
 <211> 48
 <212> DNA
 <213> Artificial Sequence

<220>

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 92-107 of the VH region (SEQ ID No 10). The primer and the template overlaps by 21 nucleotides.

<220>
<221> primer_bind
<222> (1)..(48)
<223>

<400> 16
tttgatttcc accttggtgc ctccaccgaa cgtccacgga agcgtatt 48

<210> 17
<211> 371
<212> DNA
<213> Artificial Sequence

<220>
<223> FR-patched heavy chain variable region sequence (Full DNA Sequence) formed by joining the N- and C- terminal (SEQ 19 and 22) halves at the KpeI site.

<220>
<221> V_region
<222> (1)..(371)
<223>

<400> 17
caggtgcaac tgggtggcttc cggggctgag gtaaataagc ctggggcctc agtgaaggtc 60
tcctgcaagg cttctggcta cacatttacc agttacaata tgcactgggt acggcagcct 120
cctggaaggg gcctggaatg gattggagct atttatccag gaaatggtga tactagttac 180
aatcagaaat tcaagggcaa ggccacattg actgcagaca aatcctccag cacagcctac 240
atgcagctca gcagtctgac atctgaggac tctgcggctc attactgtgc aagatcgcac 300
tacggtagta actacgtaga ctactttgac tactggggcc aaggcaccac tgttacagtc 360
tcctctgac a 371

<210> 18
<211> 123
<212> PRT
<213> Chimaera sp.

<400> 18
Gln Val Gln Leu Val Ala Ser Gly Ala Glu Val Asn Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Asn Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser His Tyr Gly Ser Asn Tyr Val Asp Tyr Phe Asp Tyr Trp

100

105

110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Asp
 115 120

<210> 19
 <211> 114
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> N-template is a synthetic sense-strand oligonucleotide encoding a
 mino acide 12-49 of the VH region (SEQ ID No. 18). The template i
 s PCR-amplified by two primers (SEQ ID No. 20 and 21)

<220>
 <221> V_region
 <222> (1)..(114)
 <223>

<400> 19
 aataagcctg gggcctcagt gaaggctctcc tgcaaggctt ctggctacac atttaccagt 60
 tacaatatgc actgggtacg gcagcctcct ggaagggggcc tggaatggat tgga 114

<210> 20
 <211> 57
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am
 ino acid 1-19 of the VH region (SEQ ID No 18). The 3' end of the
 primer overlaps with the 5'end of the template by 24 nucleotides

<220>
 <221> primer_bind
 <222> (1)..(57)
 <223>

<400> 20
 caggtgcaac tggtggcttc cggggctgag gtaaataagc ctggggcctc agtgaag 57

<210> 21
 <211> 55
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encodi
 ng amino acid 43-60 of the VH region (SEQ ID No 18). The primer
 and the template overlaps by 21 nucleotides.

<220>
 <221> primer_bind
 <222> (1)..(55)
 <223>

<400> 21
 tgtaactagt atcaccattt cctggataaa tagctccaat ccattccagg cccct 55

<210> 22
 <211> 126
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C-terminal is a synthetic sense-strand oligonucleotide encoding a
 mino acid 70-111 of the VH region (SEQ ID No 18) The template is

PCR-amplified by two primers (SEQ ID No 23 and 24)

<220>
<221> V_region
<222> (1)..(126)
<223>

<400> 22
ttgactgcag acaaatcctc cagcacagcc tacatgcagc tcagcagtct gacatctgag 60
gactctgcgg tctattactg tgcaagatcg cactacggta gtaactacgt agactacttt 120
gactac 126

<210> 23
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 57-76 of the VH region (SEQ ID No 18). The 3' end of the primer overlaps with the 5' end of the template by 21 nucleotides.

<220>
<221> primer_bind
<222> (1)..(61)
<223>

<400> 23
tgatactagt tacaatcaga aattcaaggg caaggccaca ttgactgcag acaaatcctc 60
c 61

<210> 24
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 105-123 of the VH region (SEQ ID No 18). The primer and the template overlaps by 21 nucleotides.

<220>
<221> primer_bind
<222> (1)..(59)
<223>

<400> 24
tgatcagagg agactgtaac agtggtgcct tggccccagt agtcaaagta gtctacgta 59

<210> 25
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> FR-patched light chain variable region sequence (Full DNA Sequence) formed by joining the N- and C- terminal (SEQ 27 and 30) halves at the BspEI site.

<220>
<221> V_region
<222> (1)..(321)
<223>

<400> 25
gatattcaac tcacacagtc tccatcaagt ctttctgcat ctgtggggga cagagtcaca 60


```

attacttgca gggccagctc aagtttaagt ttcatgcact ggtaccagca gaagccagga      120
tcctccccc aaccctggat ttatgccaca tccaacctgg ctccggagt ccctagtcgc      180
ttcagtgcca gtgggtctgg gaccgagttc actctcacia tcagcagttt gcagcctgaa      240
gatttcgcca cttatttctg ccatcagtg agtagtaacc cgctcacgtt cgggtgctggg      300
accaagctga ccgttctacg g                                     321

```

<210> 26
 <211> 107
 <212> PRT
 <213> Chimaera sp.

<400> 26

```

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1           5           10           15

```

```

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Leu Ser Phe Met
          20           25           30

```

```

His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
          35           40           45

```

```

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
          50           55           60

```

```

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65           70           75           80

```

```

Asp Phe Ala Thr Tyr Phe Cys His Gln Trp Ser Ser Asn Pro Leu Thr
          85           90           95

```

```

Phe Gly Ala Gly Thr Lys Leu Thr Val Leu Arg
          100          105

```

<210> 27
 <211> 129
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> N-template is a synthetic sense-strand oligonucleotide encoding a
 mino acide 9-51 of the VL region (SEQ ID No. 26). The template is
 PCR-amplified by two primers (SEQ ID No. 28 and 29)

<220>
 <221> V_region
 <222> (1)..(129)
 <223>

```

<400> 27
tcaagtcttt ctgcatctgt gggggacaga gtcacaatta cttgcagggc cagctcaagt      60
ttaagtttca tgcactggta ccagcagaag ccaggatcct cccccaaacc ctggatttat      120
gccacatcc                                     129

```

<210> 28
 <211> 45
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5' Primer is a synthetic sense-strand oligonucleotide encoding am
 ino acid 1-15 of the VH region (SEQ ID No 26). The 3' end of the

primer overlaps with the 5' end of the template by 21 nucleotides

<220>
<221>
<222>
<223>

primer_bind
(1)..(45)

<400> 28

gatattcaac tcacacagtc tccatcaagt ctttctgcat ctgtg

45

<210>
<211>
<212>
<213>

29
40
DNA
Artificial Sequence

<220>

<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 45-57. The primer and the template overlaps by 21 nucleotides.

<220>
<221>
<222>
<223>

primer_bind
(1)..(40)

<400> 29

ggactccgga agccagggttg gatgtggcat aaatccaggg

40

<210>
<211>
<212>
<213>

30
120
DNA
Artificial Sequence

<220>

<223> C-terminal is a synthetic sense-strand oligonucleotide encoding amino acid 61-100 of the VH region (SEQ ID No 26). The template is PCR-amplified by two primers (SEQ ID No 31 and 32)

<220>
<221>
<222>
<223>

V_region
(1)..(120)

<400> 30

ttcagtggca gtgggtctgg gaccgagttc actctcaca tcagcagttt gcagcctgaa

60

gatttcgcca cttatttctg ccatcagtg agtagtaacc cgctcacgtt cggtgctggg

120

<210>
<211>
<212>
<213>

31
43
DNA
Artificial Sequence

<220>

<223> 5' Primer is a synthetic sense-strand oligonucleotide encoding amino acid 54-67 of the VH region (SEQ ID No 18). The 3' end of the primer overlaps with the 5' end of the template by 21 nucleotides.

<220>
<221>
<222>
<223>

primer_bind
(1)..(43)

<400> 31

ggcttccgga gtccctagtc gcttcagtg cagtggtgt ggg

43

<210> 32
<211> 42

<212> DNA
<213> Artificial Sequence

<220>
<223> 3' Primer is a synthetic anti-sense-strand oligonucleotide encoding amino acid 94-107 of the VH region (SEQ ID No 26). The primer and the template overlaps by 21 nucleotides.

<220>
<221> primer_bind
<222> (1)..(42)
<223>

<400> 32
ccgtagaacg gtcagcttgg tcccagcacc gaacgtgagc gg

42